

CHEMICAL EXAMINATION OF SARCOCOCCA SALIGNA

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From the crude alkaloidal mixture (1.3%) obtained from cold alcoholic extract of the dried leaves of *Sarcococca saligna*, two new alkaloids have been isolated. (I) A shining crystalline base, provisionally named Salignine, has been obtained in an yield of 0.12%, has molecular formula $C_{29}H_{52}N_2O$, m.p. 229-30°C, $[\alpha]_D^{25} + 18.5$ (MeOH) and gives the following derivatives: methiodide $C_{30}H_{55}N_2OI$, m.p. 268-69°C., hydrochloride, $C_{29}H_{52}N_2O \cdot HCl \cdot H_2O$, m.p. 261-63°C., picrate m.p. 128-30°C. (decomp), Chloroplatinate m.p. 214-15 (decomp), and gold chloride m.p. 177-78°C. (decomp). (II) A white needle shaped crystalline product obtained in 0.05% yield has m.p. 136-37°C., $[\alpha]_D^{25} + 64$ (MeOH), and molecular formula $C_{25}H_{44}ON$.

Introduction

Sarcococca saligna Muel (*S. pruniformis* Lendl, Fam. Euphorbiaceae) is an ever green shrub found widely but sparsely distributed from North West Region of Pakistan (alt. 5-9000 ft.) to Bhutan; Khasia Mts. and Munnipore (alt. 4-6000 ft.) and Deccan Peninsula of India, Ceylon, Afghanistan and Sumatra.¹ The leaves of the herb enjoy considerable reputation as a remedy for different diseases and the local people use the same as a popular medicine. But as practically no literature on such an important medicinal shrub is available, the present work has been undertaken for chemical investigation of the plant. However, I.C. Chopra and K.L. Handa² reported some work on *Sarcococca pruniformis*. They obtained a crystalline product m.p. 236-38°C. ($C_{22}H_{36}ON_2$) and an orange-coloured matter m.p. 251-52°C. Present investigations of *Sarcococca saligna* reveal that its alkaloidal content is fairly high (1.2 to 1.5% on air-dried leaves). A concentrate of an alcoholic extract of the powdered dried leaves was processed in the usual way and the aqueous filtrate after basification was extracted with ether. The ether extract on concentration gave an alkaloid (I) which crystallised from acetone in shining flakes, m.p. 229-30°C. (yield 0.12%) and $[\alpha]_D^{25} + 18.5$ (MeOH). The elemental analyses were in excellent agreement with the molecular formula $C_{29}H_{52}ON_2$. It formed an orange yellow picrate m.p. 128-30°C. (decomp), methiodide m.p. 268-69°C (decomp), hydrochloride m.p. 261-63°C., chloroplatinate m.p. 214°C. and gold chloride m.p. 177-78°C.

After the removal of the alkaloid (I), the residual portion, a sticky semisolid mass, was adsorbed on a column of alumina. On elution with a mixture of benzene and petroleum ether (1:1), another alkaloid (II) m.p. 137°C. was obtained in an yield of 0.05%. Further elution with benzene gave an almost colourless semisolid

alkaloid (III) which could not be crystallised (yield 0.3%). However, it formed crystalline derivatives. From the molecular formula and other characteristic data the alkaloid (I) appears to be a new base and has been provisionally named Salignine. Work on the constitution of salignine and further studies on alkaloids II and III will form subject of subsequent papers.

Experimental

[As no difference was observed in the products of fresh and dried plant, the latter was used for all necessary extractions].

Air dried powdered leaves of *Sarcococca saligna* (500 g.) were extracted with cold freshly distilled alcohol (3 l.) for three to four days or refluxed for about twelve hours. After complete extraction and filtration, the residual leaves were rinsed with fresh solvent and the combined alcoholic extracts were concentrated under reduced pressure. The concentrate was treated with acidulated water and allowed to stand. When the precipitated green-coloured matter settled down, the mixture was filtered through a charcoal bed on a Buchner funnel by suction. The reddish clear filtrate was basified with ammonia and extracted either with benzene, ether or petroleum ether. The extract was dried over anhydrous sodium sulphate and the solvent was removed by distillation and finally under vacuum. The residual sticky semisolid mass (yield 1.3%) was worked up for the isolation and characterisation of the various components as detailed below:

(A) *Alkaloid (I), Salignine*.—The crude mixture of alkaloids (6.2 g.) was dissolved in a minimum quantity of hot acetone. The solution on cooling overnight, gave a crystalline product. It was filtered and washed with a small quantity of acetone and the combined filtrate on gradual concentration and subsequent cooling gave two successive crops of crystals of the same product (total yield, 0.12%). It was recrystallised from acetone or

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alcohol in shining white flakes, m.p. 229-30°C. The product was subjected to ascending unidimensional paper chromatography using butanol: acetic acid: water (4:3:1) as the irrigating mixture. Spraying with Dragendorff's reagent produced a clean single spot showing it to be a homogeneous product. Its homogeneity was also verified by thin layer chromatography.

Some of the properties of the alkaloid have been determined and the following values were recorded, m.p. 229-30°C., mol. wt. 446 (Rast camphor method). $[\alpha]_D^{25} + 18.5$, R_f 0.88 (butanol, acetic acid and water, 4:3:1, paper chromatogram Whatman No. 1); Found: C, 78.36; H, 11.47; N, 6.57; O, 3.6%; C-methyl, 7.5; NMe, 11.15%. $C_{29}H_{52}ON_2$ requires C, 78.55; H, 11.5; N, 6.32; O, 3.61%; 3C-methyl, 7.68; 2NMe, 10.93%. Infra-red spectra of the base are reproduced in Fig. 1.

chloride was added drop by drop until the mixture was faintly acidic to litmus. The solvent was removed under vacuum in a desiccator. The white product was crystallised repeatedly and finally melted at 261-63°C. Found: C, 70.81; H, 10.59; Cl, 7.20; N, 5.05; O, 5.9%; $C_{29}H_{52}N_2O$, HCl, H_2O requires: C, 69.9; H, 10.9; Cl, 7.20; N, 5.7; O, 6.37%. These results also show that the base reacts with one molecular proportion of hydrochloric acid. The hydrochloride appears to contain one molecule of water presumably as water of crystallisation.

(c) *Picrate*: The base (0.2 g.) was dissolved in dilute acetic acid. An aqueous solution of picric acid was added to the solution of the base until precipitation was complete. The yellow precipitate was filtered, washed and freed from water as completely as possible by suction. The residue was dissolved in hot acetone and repeatedly

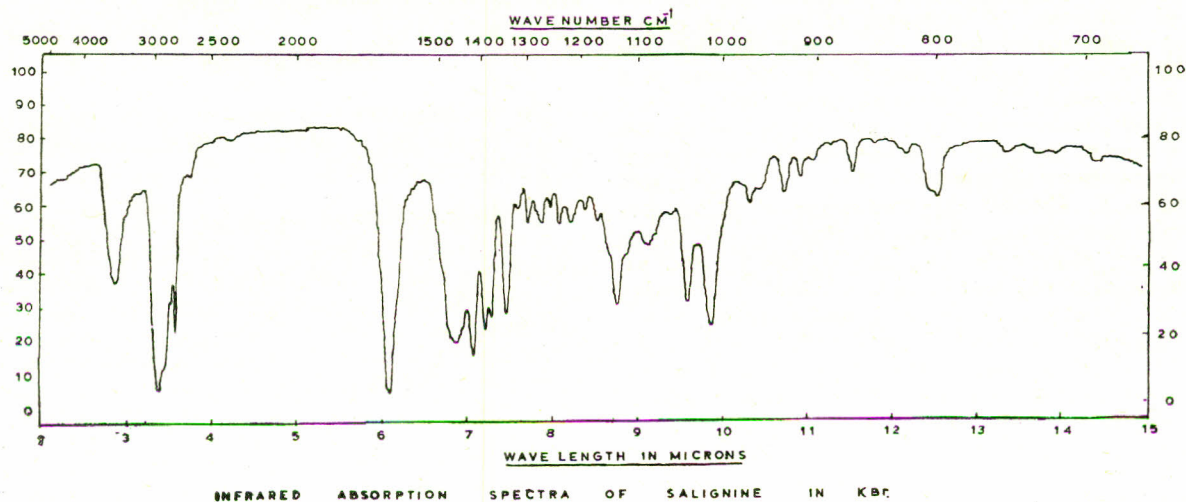


Fig. 1.

Preparation of Derivatives. (a) *Methiodide*: The alkaloid (0.1 g.) was dissolved in dry benzene (50 ml.). Methyl iodide (15 ml.) was added and the mixture was kept in a dark place in a corked flask for about 6 hours. The white crystalline precipitate was filtered and washed with a fresh quantity of benzene. The methiodide was twice recrystallised from alcohol, m.p. 268-69°C. Found: C, 61.36; H, 9.12; N, 4.87; I, 21.90; O, 2.75%. $C_{30}H_{55}N_2OI$ requires: C, 61.54; H, 9.23; N, 4.78; I, 21.7; O, 2.75%. The results account for one molecular proportion of the base and methyl iodide being involved in the reaction.

(b) *Hydrochloride*: The base (0.2 g.) was dissolved in methyl alcohol and methanolic hydro-

crystallised from the same solvent. Beautiful yellow needle shaped crystals m.p. 128-30°C. (decomp) were obtained.

(d) *Chloroplatinate*: The alkaloid (0.15 g.) dissolved in dilute hydrochloric acid was treated with chloroplatinic acid ($H_2PtCl_6 \cdot xH_2O$). The reddish yellow crystalline precipitate was immediately filtered, washed free of chloride and dried in a desiccator over concentrated sulphuric acid under vacuum. The chloroplatinate melted at 214°C. (decomp).

(e) *Gold Chloride*: The base (0.15 g.) was dissolved in dilute hydrochloric acid. On the addition of an aqueous solution of chloroauric acid, a

yellow crystalline precipitate was obtained which was immediately filtered and washed repeatedly to remove the chloride iron. Even prolonged washing did not make the filtrate completely free from chloride showing that gold chloride of the base is sparingly soluble in cold water. After drying over concentrated H_2SO_4 for a few hours, it had m.p. $177-78^\circ C.$ (decomp).

Bromination of Salignine.—To the chloroform solution of the alkaloid, bromine in chloroform was added drop by drop until the mixture retained faint colour of bromine. On removal of the solvent under vacuum a sticky mass was obtained. On treatment with different solvents, it did not give any crystalline product.

Reaction with Potassium Permanganate.—The base (0.2 g.) was dissolved in acetic acid and treated with a cold dilute potassium permanganate solution (N/10) until a faint pink colour persisted. The mixture after basification was extracted with chloroform, dried over anhydrous sodium sulphate and on concentration gave a white product which after crystallisation from alcohol or acetone, melted at $208^\circ C.$

(B) *Alkaloid (II).*—A portion (0.2 g.) of the mother liquor after the separation of Salignine was completely freed from solvent, taken in benzene (25 ml.) and subjected to chromatographic resolution on a column ($8 \times 1'$) of standardised Brockmann alumina. Benzene (25 ml.) was then cautiously introduced into the column when uniform absorption was ensured. The column was developed with petroleum ether and then eluted with a mixture of benzene and petroleum ether (1:1). The eluate was collected in 50 ml. portions. The first fraction gave the alkaloid (II); the second and third lots also gave a small quantity of the same product. It was purified by repeated crystallisation from acetone. White needle shaped crystals m.p. $136-37^\circ C.$ were obtained (yield, 0.05%). The homogeneity of the product was ascertained by ascending unidimensional paper chromatography. R_f 0.95 (butanol, acetic acid and water, 4:3:1, paper chromatogram Whatman No. 1). Found: C, 80.22; H, 11.43; N, 4.12; O, 4.23; calculated for $C_{25}H_{44}ON$; C, 80.21; H, 11.73; N, 3.74; O, 4.27.

(C) *Isolation of Alkaloid (III).*—After the separation of the alkaloid (II), when the eluate ceased to give any test for alkaloid, the column was eluted with benzene and 50 ml. lots were collected. The fractions were separately worked out, but gave a sticky glassy mass and no crystalline substance. The middle fractions were proved homogeneous by

ascending unidimensional paper chromatography, R_f 0.86, (butanol, acetic acid and water, 4:3:1, paper chromatogram Whatman No. 1). Attempts to crystallise the residue in various solvents under different conditions did not succeed. However, the product gave crystalline methiodide m.p. $258-59^\circ C.$, hydrochloride m.p. $251-52^\circ C.$

Discussion

The base Salignine, $C_{29}H_{52}N_2O$ contains two nitrogen atoms one of which is reactive to a molecular proportion of methyl iodide. The nitrogen atom involved in the reaction appears to be basic and tertiary in character. The analysis of the hydrochloride also gives one chlorine atom and this tends to support the above presumption. The hydrochloride is associated with a molecule of water and appears to have the formula $C_{29}H_{52}N_2O, HCl, H_2O$. The nature of the other nitrogen atom has not yet been ascertained. Analysis shows that there are three carbon bound and two nitrogen bound methyl groups present in the molecule.

As no hydroxy, ketone and aldehyde derivatives could be prepared, the nature of occurrence of the oxygen atom could not be ascertained. The infra-red absorption bands (Fig. 1) at 12.6μ and 10.34μ suggest double bonds and those at 9.9 and 9.65 suggest an ortho substituted benzene. The small bands at 7.6 to 8.4 indicate a number of methylene groups possibly in a chain or saturated ring and the doublet at 7.3 indicates an isopropyl. These assignments are possibilities and in no way final. Their confirmation will await further chemical investigation which are in progress.

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